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IAP5 Rec'd PCT/PTO 30 MAR 2006

COURTESY COPY OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY WITH ANNEXES CONTAINING PAGES 82-85 TO BE SUBSTITUTED FOR ORIGINAL PAGES 82-85, CLAIMS 1-41 TO BE SUBSTITUTED FOR ORIGINAL CLAIMS 1-62, AND FIGURES 5 AND 7 TO BE SUBSTITUTED FOR ORIGINAL FIGURES 5 AND 7 FOR EXAMINATION IN THIS CASE

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P810PC00	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/DK2004/000659	International filing date *(day/month/year)* 29.09.2004	Priority date *(day/month/year)* 30.09.2003
International Patent Classification (IPC) or national classification and IPC C07K5/00, C07K7/00, C07K14/00, G01N33/68, A61K38/04, A61K38/17		
Applicant ENKAM PHARMACEUTICALS A/S et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 13 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.08.2005	20.03.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Moonen, P Telephone No. +31 70 340-8991

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000659

IAP5 Rec'd PCT/PTO 30 MAR 2006

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-81, 86-90	as originally filed
82-85	received on 29.08.2005 with letter of 26.08.2005

Sequence listings part of the description, Pages

1-23	as originally filed

Claims, Numbers

1-41	received on 20.02.2006 with letter of 17.02.2006

Drawings, Sheets

1/63-55/63, 57/63, 59/63-63/63	as originally filed
56/63, 58/63	received on 29.08.2005 with letter of 26.08.2005

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000659

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☒ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-6, 40 completely; 8-39 and 41 partially

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 8-39 and 41 (partially) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-6 and 40 (completely); 8-39 and 41 (partially) concerning inventions 2-4

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☒ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 8-39 and 41 (partially), concerning invention 1 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8-39 and 41 (partially)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	8-39 and 41 (partially)
Industrial applicability (IA)	Yes:	Claims	8-39 and 41 (partially)
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Reference is made to the following documents:

D1: RAO Y ET AL: "Identification of a peptide sequence involved in homophilic binding in the neural cell adhesion molecule NCAM" JOURNAL OF CELL BIOLOGY, ROCKEFELLER UNIVERSITY PRESS, NEW YORK, US, US, vol. 118, no. 4, August 1992 (1992-08), pages 937-949

D2: DATABASE HTTP://WWW [Online] 2002, KASPER ET AL.: "Extracellular modules of the cell adhesion molecules", retrieved from HTTP://WWW-HASYLAB.DESY.DE/SCIENCE/ANNUAL_ REPORTS/2002_REPORT/PART2/CONTRIB/72/7824. PDF

D3: ATKINS A R ET AL: "Solution structure of the third immunoglobulin domain of the neural cell adhesion molecule N-CAM: can solution studies define the mechanism of homophilic binding?" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 311, no. 1, 3 August 2001 (2001-08-03), pages 161-172

D4: RONN L C B ET AL: "IDENTIFICATION OF A NEURITOGENIC LIGAND OF THE NEURAL CELL ADHESION MOLECULE USING A COMBINATORIAL LIBRARY OF SYNTHETIC PEPTIDES" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 17, October 1999 (1999-10), pages 1000-1005

D5: SOROKA VLADISLAV ET AL: "Induction of neuronal differentiation by a peptide corresponding to the homophilic binding site of the second Ig module of the neural cell adhesion molecule" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 27, 5 July 2002 (2002-07-05), pages 24676-24683

D6: KRISTIANSEN L V ET AL: "Homophilic NCAM interactions interfere with L1 stimulated neurite outgrowth" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 464, no. 1-2, 24 December 1999 (1999-12-24), pages 30-34

D7: JENSEN PETER HOLME ET AL: "Structure and interactions of NCAM modules 1 and 2, basic elements in neural cell adhesion" NATURE STRUCTURAL BIOLOGY, vol. 6, no. 5, May 1999 (1999-05), pages 486-493, XP002315063 ISSN: 1072-8368

D8: KASPER CHRISTINA ET AL: "Structural basis of cell-cell adhesion by NCAM" NATURE STRUCTURAL BIOLOGY, vol. 7, no. 5, May 2000 (2000-05), pages 389-393

D9: WO 00/18801 A2 (ROENN, LARS, CHRISTIAN, B; BOCK, ELISABETH; HOLM, ARNE; OLSEN, MARIANN) 6 April 2000 (2000-04-06)

D10 Huang et al. Biopolymers **43** (1997) 367-382

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Present **claims 8-39 and 41** partially relate to an extremely large number of possible uses of compounds, methods and compounds per se. In fact, the claims contain so many options and variables, that a lack of clarity (and conciseness) within the meaning of Article 6 PCT arose to such an extent that a meaningful full search of the claims was rendered impossible.
Consequently, the search was and therefore also this opinion is restricted to those parts of the application which do appear clear and concise, namely the compounds and methods of **invention 1** when referring to polypeptides with specified sequences (**SEQ ID NOs: 1-3, 40 and 41**), and not to undefined fragments variants thereof.

Re Item IV

Lack of unity of invention

The separate inventions/groups of inventions are:

Invention 1: Claims 8-39 and 41, partially
Use of compounds, capable of binding to the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between Ig1 and Ig3 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.
Invention 2: Claims 8-39 and 59, partially
Use of compounds, capable of binding to the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between Ig2 and Ig3 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.
Invention 3: Claims 8-39 and 41, partially
Use of compounds, capable of binding to the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between two Ig2 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.

Invention 4: Claims 1-6 and 40
Crystals of a polypeptide comprising the Ig1-Ig2-Ig3 module of NCAM, their use and method of crystallisation.
Invention 5: Present Claim 7 completely
Method for selecting a candidate compound based on a structural model of the Ig1-Ig2-Ig3 modules of NCAM, obtainable eg from the soluble or crystalline polypeptide.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

Introduction:
Two structurally related CAMs, the neural cell adhesion molecule (NCAM) and L1, are prominent members of the immunoglobulin superfamily, and are also known to interact with each other (Kristiansen et al. 1999; D6). Recombinant Ig modules 1, 2 and 3 of NCAM, involved in homophylic NCAM binding (see abstract of **D6**), gave complete inhibition of L1 induced neurite outgrowth. NCAM engages also in a calcium-independent, homophilic binding originally suggested to depend on a reciprocal interaction between the third Ig-module, or on all five Ig-modules of two opposing NCAM molecules; later it has been shown that also the first and the second Ig-modules of NCAM bind to each other in a so-called double reciprocal interaction (eg Atkins et al. Fig. 1; **D3**). Using NMR spectroscopy the 3D-structure of the first and second Ig-module of NCAM was recently solved, and putative reciprocal binding sites were identified, providing a structural model of an anti-parallel binding between the two Ig-modules (Jensen et al.; **D7**); crystallisation and structural data of high quality crystals of NCAM Ig1-Ig2 were provided by Kasper et al. ((2000); **D8**).

Motivation for the split into five inventions:
In the present invention, the structural work has been extended (see Kasper et al. (2002); **D2**) in comparison to **D8** by elucidating the 3D structure of the Ig1-Ig2-Ig3 module of NCAM; D2 mentioned already the crystallization of the Ig1-2-3 triple-domain and the importance of Ig3 in homophylic binding (see also Soroka et al (2000), **D5**, in particular the introduction when citing references 5 and 6). The solution structure of the Ig3 module had already been disclosed in **D3**, as well as the expression of recombinant chicken IgI-III NCAM and a mutant (Phe19) thereof, establishing a residue important in Ig1-2

dimerization. 3D structural studies can be standardly carried out, eg as described earlier in the prior art to find parts of the modules interacting with each other, and to propose compounds interfering with the contact points; in addition, the model can be used to evaluate the binding of peptides known to be involved in homophylic binding (e.g. peptide P5 disclosed by Rao et al. (**D1**), derived from chicken Ig3 and with sequence KYSFNYDGSELIKKVDKSDE (see Table III), has already been referred to in relation to modulation of NCAM homophylic binding; this peptide, as part of chicken Ig3, has the corresponding sequence SEQ ID NO:20 of rat Ig3 as presently mentioned in the description (see Figure 11 of D1); in **D5** a peptide P2 derived of the Ig2 module is disclosed, P2 with sequence GRILARGEINFK (see eg Figure 9), being involved in Ig1 binding, neurite outgrowth and inhibiting cell aggregation (see also **WO 00/18801**, SEQ ID NO:23); in **D4** (Ronn et al.) a combinatorial library was used to find a synthetic, neuritogenic peptide C3, with sequence ASKKPKRNIKA, binding to Ig1 at a site different from the binding site of the NCAM Ig2 module; see also WO 00/18801, SEQ ID NO:1). WO 00/18801, in particular page 24 line 18 and further, discloses SEQ ID NO:26 with sequence GEISVGESKFFL, an Ig1 peptide binding apparently to the part of the homophilic binding site of NCAM Ig1-Ig2 which is constituted by the Ig2 domain and identical to SEQ ID NO:19 of the present application.

Thus a method of modulating outgrowth of neurites presenting NCAM with different NCAM ligands interacting with homophylic binding of NCAM, in particular involving the Ig1 and Ig2 modules,was already known, as well as crystals and structure of the Ig1-Ig2 fragments of the cross-like, anti-parallel Ig1-Ig2 dimer (Kasper et al 2000); furthermore, the solution structure of the Ig3 module had been disclosed as well as the role of Ig3 in homophilic binding. The crystallisation of Ig1-Ig2-Ig3 has been suggested and different peptides were known to interfere with homophilic binding (reference is made to the known SEQ ID NO:19 as referred to in the present application), as well as methods to find additional peptide sequences (by rational design based on structure or by combinatorial libraries).

Conclusion:

It is therefore considered that a special technical link between the inventions I-III, the crystals of Ig1-Ig2-Ig3 or selection methods is absent. According to Rule 13 PCT, a group of inventions is only linked to form a single inventive concept where there is a technical relationship among the inventions that involves at least one common or corresponding

special technical feature that defines the contribution which each claimed invention, considered as a whole, makes over the prior art. No such a technical relationship for the listed five inventions is identifiable in view of the cited prior art with respect to the structural studies to obtain of the first three NCAM modules and the peptides relevant to several types of NCAM homophylic binding. Accordingly, the claims of these five inventions are not so linked by a special, new and inventive technical feature under PCT Rule 13 and therefore lack unity of invention is present.

To be noted is that further non-unitarily linked subject-matter appears to be present within present invention 1 on the basis of the fact that SEQ ID NO:20 was obvious to the skilled person. Each specified peptide and its use as a ligand appears therefore to represent a separate invention.

The applicant decided to pay one additional search fee under protest with respect to invention 5. After the Chapter II request, the Applicant was requested to either limit the application to invention 1 or 5, or to pay a further examination fee for the second searched invention. The Applicant decided not to answer to this invitation, and the **IPER** (International preliminary examination report) is therefore established **for the first invention only.**

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Newly filed claim 29 has been amended: however, it is noted that this claim is not considered to be of a second medical use-type, as it does not specify a particular **medical therapy** for which the manufactured medicament will be of use; it only specifies which cells the compound should modulate.

2. The present invention does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of claims 8-39 and 41 (as far as invention 1 is concerned) does not involve an inventive step (Rule 65(1)(2) PCT).

   The peptides of claim 8, considered to belong to invention 1 and partially searched

(the claim has an undefined scope by referring to "a fragment or a variant of said sequence"), are for example the peptides having SEQ ID NO:40 and 41 (being a part of Ig1; present claims 27-28). The other sequences belonging to invention 1 have been submitted to be SEQ ID Nos 1-3 (description page 84, last paragraph).

3. The peptides having sequences like SEQ ID NO:40 and 41 are considered to have been obvious to the skilled person in view of the combination of documents **D2** (see the top of page 2) and **D3.** The consideration of peptide sequences with respect to binding sites follow in an obvious way from the 3D-structure. At present, it has to be noted that nothing indicates that the skilled person was not in the position to repeat the crystallisation indicated in D2; with respect to D3 it is noted that this document leaves several options open with respect to the interacting Ig domains, and it concludes (in the abstract) that in solution different interactions are possible than that occur on the cell surface, *eg* the interactions in crystals may come closer to the true domain interactions. The reasoning about obviousness applies also to the pharmaceutical use, as this use was already suggested in the prior art for this type of peptides. Said last mentioned peptides appear also to lack the right of priority, making the **P,X document** of the search report (the publication of the present invention) available as a citable document.

4. With respect to the peptides with sequences SEQ ID NO:1 and 2 (part of Ig1) and SEQ ID NO:3 (part of Ig3), it is additionally noted that these peptide have not been demonstrated to **bind** to a NCAM homophylic binding site composed of Ig1/Ig3 modules of NCAM. It is therefore not clear if the technical problem is likely to be solved for these peptides. This demonstration is necessarry for the acknowledgement of the inolvement of an inventive step.

****

The X-ray structure of NCAM Ig1-2-3 was determined to 2.0 Å resolution (see Table 1 of Figure 1). In the structure of Ig1-2-3, the Ig1 and Ig2 modules are positioned in an extended conformation with Ig3 oriented at an angle of approximately 45° to the Ig1-Ig2 axis (Figure 3). The linker regions between Ig1-Ig2 and between Ig2-Ig3 are short and comprise only two (Lys98 – Leu99) and one (Asn190) residues, respectively. The overall structure of the Ig1 and Ig2 modules is very similar to the previously determined Ig1-2 structure (Kasper et al., 2000) with root mean square deviations (r.m.s.d.) of 0.7 (96 Cα atoms) and 0.8 Å (93 Cα atoms), respectively. In the Ig1-2-3 structure, the tilt angle between Ig1 and Ig2 is 11° and thereby differs by 13° compared to the Ig1-2 structure.

The 98-residue Ig3 module of rat NCAM adopts the topology of an intermediate type 1 (I1) set Ig module (Casasnovas et al., 1998). In the Ig3 module, the classical β-sandwich consists of two β-sheets with a total of nine β-strands (Figure 3B). The A, B, D, and E β-strands make up one sheet and the A', C, C', F, and G β-strands the second sheet. A cysteine bridge Cys216 – Cys269 connects the two β-sheets. All strands are anti-parallel except for the A' strand, which runs parallel to the C-terminal part of the G strand. Ig3 contains one site for N-linked glycosylation at Asn203 positioned in the A' strand. The E-F loop (residues Lys261 – Asp263) forms a 3_{10} α-helical turn. The overall structure of rat Ig3 is similar to the structure of chicken Ig3 (Atkins et al., 2001) with r.m.s.d. of 1.65 Å (95 Cα atoms).

Parallel interactions between Ig modules

Several characteristic interactions are observed in the structure of the NCAM Ig1-2-3 fragment which may be divided into two groups: Interactions where the long axes (N- to C-terminus) of two interacting Ig1-2-3 molecules are oriented in a parallel manner and interactions where the long axes are oriented in an anti-parallel manner. One parallel interaction and three major anti-parallel interactions are observed in the crystal.

The parallel, cross-like dimer interaction of NCAM Ig1-2-3 involves the Ig1 and Ig2 modules (Figure 5). The total buried surface area of this interface is 1594 $Å^2$ (per dimer), which is similar to that previously observed in the Ig1-2 cross-like dimers (Kasper et al., 2000). The most prominent feature of the Ig1-to-Ig2 interaction is the intercalation of two aromatic residues of Ig1, Phe19 and Tyr65, into hydrophobic pockets formed by Ig2 residues (Figure 5A), which was also observed in the Ig1-2 structure. However, a tighter Ig1 to Ig2 binding interface is observed in the Ig1-2-3

structure, where the hydroxyl group of Tyr65 forms a direct hydrogen bond (H-bond) with Glu171, instead of a water-mediated H-bond as observed in Ig1-2. Tyr65 also makes three H-bonds to the side chains of Lys133, Glu171, and Arg173. Arg173 forms part of the Ig2 hydrophobic pocket and makes two H-bonds to Thr63. The parallel orientation of the Arg173 and Phe19 side chains and the distance between the N□ι atom of the guanidinium group of Arg173 and the Cζ atom of the benzene ring of Phe19 (3.4 Å) suggest a cation-π interaction between these two residues (Flocco and Mowbray, 1994).

Dynamic Light Scattering (DLS) measurements showed that deglycosylated Ig1-2-3 forms a single species of molecules in solution with a molecular weight of ~78 kDa, corresponding to a dimer. In order to demonstrate that Ig1-2-3 dimerization is mediated by the observed Ig1 to Ig2 binding, we produced a mutant of Ig1-2-3 (Ig1-2-3mut) containing three Ala substitutions: E11A, E16A, and K18A. These mutations have previously been shown to completely abolish dimerization of the Ig1-2 NCAM fragment in solution (Jensen et al., 1999). In the present structure Glu11 and Glu16 form intramolecular salt bridges, respectively, with Arg177 and Lys98 from the Ig1 to Ig2 linker region (not shown). These salt bridges probably contribute to the proper orientation of Ig1 with respect to Ig2 and therefore are important for the Ig1-to-Ig2 interaction. Lys18 forms an H-bond with the carboxyl group of Arg177 from the Ig2 module stabilizing the Ig1-Ig2 interaction (Figure 5A). Lys18 is located near Phe19, which is the critical residue for the Ig1-to-Ig2 interaction as it was clearly demonstrated earlier (Atkins et al., 2001). Therefore, disruption of the Lys18 - Arg177 H-bond may affect the orientation of Phe19 leading to elimination of the Ig1-to-Ig2 interaction. The molecular weight of the Ig1-2-3mut fragment was determined by DLS to be ~34 kDa, indicating a monomer. This confirms that Ig1-2-3 dimerization is mediated by Ig1-to-Ig2 binding.

Parallel (*cis*) interactions are not uncommon among cell adhesion molecules. Thus, *cis* dimerization has been demonstrated for the cell adhesion molecules C-CAM1, C-CAM2, ICAM-1, nectin-2α, and JAM belonging to the Ig superfamily (Hunter et al., 1996; Casasnovas et al., 1998; Miyahara et al., 2000; Kostrewa et al., 2001) as well as for N-, E-, and C- cadherins (Shapiro et al., 1995; Takeda et al., 1999; Brieher et al., 1996). It was shown that the dimeric form of C-cadherin is capable of adhesion, whereas the monomeric form is not (Brieher et al., 1996).

Anti-parallel interactions between Ig modules

An anti-parallel interaction takes place between the Ig2 and Ig3 modules of two Ig1-2-3 molecules, thereby forming arrays of Ig1-2-3 dimers (Figure 4A,B). Ig2 of one molecule binds to Ig3 of a second molecule, and *vice versa* (Figure 3B). The residues involved are 112-115, 143-146, and 158-161 from the B-strand, CD-loop/D-strand, and E-strand of Ig2, and residues 200-205, 261, and 278-289 from the A'-strand, EF-loop, and G-strand of Ig3. A central element of this interaction is the intercalation of the side chain of Phe287 from Ig3 into a hydrophobic pocket formed by the side chains of Val145, Arg146, and Arg158 of the Ig2 module and Lys285 from Ig3. Arg158 is also involved in water-mediated hydrogen bonding to residues Lys261 and Ala288, and Gly159 makes a direct H-bond to Asn203.

The crystal packing leaves room for glycosylation at Asn203. In order to accommodate N-linked glycosylation at this site, the side chain of Asn203 has to adopt another rotamer conformation. Thereby, the carbohydrate will point away from the binding site and towards a solvent channel in the crystal, and consequently Asn203 will not interfere with Ig2-Ig3 interactions. An interaction between the two Ig3 modules is observed at the interface, as Gln196 makes a water-mediated H-bond with Gln278. The total buried surface of the Ig2-to-Ig3 interface is 1407 $Å^2$ per dimer. According to Janin (1997), the probability of finding a non-specific interface of the size of the Ig2-to-Ig3 contact is only 1.9%.

Another anti-parallel interaction between two Ig1-2-3 molecules is formed between two Ig2 modules (Figure 4C,D). This interaction involves residues 103-121 and 150-158 of the AA'-loop/A'-strand/A'B-loop and the DE-loop/E-strand and has the total buried surface of 958 $Å^2$ per dimer (Figure 4C). Here, the central residue appears to be Glu114, which makes two H-bonds to Ser151 (side chain and backbone). Apart from an extensive hydrogen-bonding network, especially through water molecules, Val117, Val119, Leu150, and Tyr154 of both Ig2 modules form a number of hydrophobic contacts with each other at the Ig2-to-Ig2 interface (not shown).

A slightly smaller anti-parallel interaction (858 $Å^2$ of total buried surface per dimer) is formed between the Ig1 and Ig3 modules (Figure 4C,D), involving residues 32-47 and 76-88 from the C-strand/CC'-loop/C'-strand/C'D-loop and F-strand/FG-loop/G-strand in Ig1, and residues 198, 213-223, and 248-253 from the A-strand, B-strand/BC-loop, and D-strand/DE-loop in Ig3 (Figure 5D). Arg198 and Asp249 form direct H-bonds to the backbone oxygen atoms of Ala81 and Glu82 and two salt bridges with Lys76, respectively. Additionally, one water-mediated H-bond is formed between Lys42 and Asp250, one between Ser44 and Gly220, and two between

Ser44 and Glu223. The conserved Phe36 and Phe221 are packed against Asp249 and Gln47, respectively. Together two Ig1-to-Ig3 interaction sites and one Ig2-to-Ig2 site make up a predominant contact between Ig1-2-3 dimers in the crystal (2654 Å^2) forming the second array of Ig1-2-3 dimers (Figure 4C,D) perpendicular to the Ig2-to-Ig3-mediated array (Figure 2A,B). Contact areas of similar sizes have been found in other CAMs. *Cis* dimers of human ICAM-1 and mouse JAM have 1100 Å^2 and 1200 Å^2 of total buried surface area (per dimer), respectively (Casasnovas et al. 1998; Kostrewa et al., 2001), whereas *trans* dimers of rat CD2 and chicken axonin-1/TAG-1 have even larger contact areas of 1300 Å^2 and 2000 Å^2 (Jones et al., 1992; Freigang et al., 2000).

Ig3 inhibits NCAM-dependent neurite outgrowth

NCAM-NCAM interaction is known to induce neurite outgrowth from NCAM-expressing PC12-E2 cells grown on a confluent monolayer of NCAM-expressing fibroblasts (Kolkova et al., 2000). Inhibition of the NCAM-NCAM interaction will therefore inhibit neurite outgrowth in PC12-E2 cells.

In order to examine the biological significance of the observed Ig1-to-Ig3 and Ig2-to-Ig3 contacts in the structure of NCAM Ig1-2-3, we tested the inhibitory effect of the recombinant Ig3 module on NCAM-NCAM adhesion. Furthermore, we prepared two Ig3 mutants containing mutations of the residues R198A, D249G, E253A (Ig3mut1) of the Ig1-to-Ig3 contact site (see Figure 5D) and K285A, F287A (Ig3mut2) of the Ig2-to-Ig3 contact site (see Figure 5B). In Figure 4 it can be seen that the wildtype Ig3 module (Ig3wt) indeed has an inhibitory effect, whereas both mutants are inactive, thereby strongly supporting that both the Ig1-to-Ig3 and Ig2-to-Ig3 contact sites are participating in homophilic interactions.

A similar co-culture test-system of NCAM-expressing chicken retinal ganglion cells grown on top of NCAM-140-transfected mouse L-cells has been successfully used to demonstrate a disruptive effect of mutations in the Ig3 module homophilic binding site (Ig1-to-Ig3 binding site in the present work) as well as to show an inhibition of neurite outgrowth by synthetic peptides representing this homophilic binding site (Sandig et al. 1994).

Interaction interface peptides inhibit neurite outgrowth

It has previously been demonstrated that peptides representing homophilic binding sequences from Ig3 and Ig2 modules of NCAM inhibit NCAM-mediated cell

Claims

1. A crystal of a polypeptide comprising the Ig1-2-3 module of NCAM, said polypeptide comprising amino acid residues 1 to 289 of SEQ ID NO: 44, wherein said crystal comprises atoms arranged in a spatial relationship represented by the structure co-ordinates of Table 2 (Figure 2) or by coordinates having a root mean square deviation therefrom of not more than 2.5 Å.

2. The crystal according to claim 11, wherein the polypeptide consists of amino acid residues 1 to 289 of SEQ ID NO: 44 and an extra amino acid sequence of 1 to 4 amino acids residues.

3. The crystal according to claim 11, wherein said crystal diffracts X-rays for determination of atomic co-ordinates to a resolution of at least 4 Å.

4. The crystal according to claim 11, wherein the crystal effectively diffracts X-rays for the determination of the atomic coordinates to a resolution at most 5. 0 Å.

5. The crystal according to claims 14 or 15, wherein the crystal effectively diffracts X-rays for the determination of the atomic coordinates to a resolution 1. 5 Å.

6. The crystal according to claim 11, wherein said crystal has unit cell dimensions of a=51.5 Å, b=108.5 Å, c= 149.0 Å, alpha=90°, beta=90°, gamma=90°.

7. A method for selecting a candidate compound capable of modulating differentiation, adhesion and/or survival of NCAM presenting cells by modulating the interaction of

   i) the Ig1 module of one individual NCAM molecule with the Ig3 module of another individual NCAM molecule, and/or

   ii) the Ig2 module of one individual NCAM molecule with the Ig3 module of another individual NCAM molecule, and/or

   iii) the Ig2 module of one individual NCAM molecule with the Ig2 module of another individual NCAM molecule,

said method comprising the steps of

   a) providing a crystalline polypeptide according to claim 1,

b) generating a structural model of the Ig1-2-3 module of NCAM of (a) by using the computer modelling techniques;

c) in-silico evaluating compounds for the capability of

i) binding to the Ig1 module of NCAM at the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules, and thereby mimicking and/or modulating the interaction between the Ig1 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

ii) binding to the Ig3 module of NCAM at the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig1 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iii) binding to the Ig2 module of NCAM at the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules, and thereby mimicking the interaction between Ig2 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

iv) binding to the Ig3 module of NCAM at the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules, and thereby mimicking and/or modulating the binding between the Ig3 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules, and/or

v) binding to the Ig2 module of NCAM at the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules, and thereby mimicking and/or modulating the interaction between the Ig2 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules,

by using the structural model of the Ig1-2-3 module of NCAM of (b);

d) selecting a candidate compound capable of at least one interaction of (c), and

e) testing the candidate compound of (d) in an in vitro assay for the capability of modulating differentiation, adhesion and/or survival of NCAM presenting cells, said assays comprising at least one NCAM presenting cell, and /or

f) testing the candidate compound of (d) in an assay comprising evaluating the capability of the compound of at least one interaction of (b) by contacting the compound with at least one individual fragment of an NCAM molecule, said fragment comprising a sequence of consecutive amino acid residues

corresponding to the sequence of the Ig1-2-3 module of NCAM comprising residues 1 to 289 of the sequence set forth in SEQ ID NO: 44.

8. A compound capable of binding to the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules, wherein said compound is capable of

i) binding to the Ig1 module of NCAM at said NCAM homophylic binding site, and thereby mimicking and/or modulating the interaction between the Ig1 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules of opposing contacting cells, and/or

ii) binding to the Ig3 module of NCAM at said NCAM homophylic binding site, and thereby mimicking and/or modulating the interaction between the Ig3 and Ig1 modules of NCAM, wherein said modules are from two individual NCAM molecules of opposing contacting cells, and/or

iii) binding to the Ig2 module of NCAM at said NCAM homophylic binding site, and thereby mimicking the interaction between Ig2 and Ig3 modules of NCAM, wherein said modules are from two individual NCAM molecules of opposing contacting cells, and/or

iv) binding to the Ig3 module of NCAM at said NCAM homophylic binding site, and thereby mimicking and/or modulating the binding between the Ig3 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules of opposing contacting cells, and/or

v) binding to the Ig2 module of NCAM at said NCAM homophylic binding site, and thereby mimicking and/or modulating the interaction between the Ig2 and Ig2 modules of NCAM, wherein said modules are from two individual NCAM molecules of opposing contacting cells,

said compound being a peptide sequence identified as SEQ ID NO: 1, 2, 3, 4, 7, 10, 11, 12, 13, 14, 16, 17, 18, 40 or 41, or being a fragment or a variant of said sequence, wherein said peptide sequence is selected by the method according to claim: 20.

9. The compound according to claim 8, said compound having the amino acid sequence WFSPNGEKLSPNQ (SEQ ID NO: 1).

10. The compound according to claim 8, said compound having the amino acid sequence YKCVVTAEDGTQSE (SEQ ID NO: 2).

11. The compound according to claim 8, said compound having the amino acid sequence TLVADADGFPEP (SEQ ID NO: 3).

12. The compound according to claim 8, said compound having the amino acid sequence QIRGIKKTD (SEQ ID NO: 4).

13. The compound according to claim 8, said compound having the amino acid sequence DVR (SEQ ID NO: 5).

14. The compound according to claim 8, said compound having the amino acid sequence RGIKKTD (SEQ ID NO: 6).

15. The compound according to claim 8, said compound having the amino acid sequence DVRRGIKKTD (SEQ ID NO: 7).

16. The compound according to claim 8, said compound having the amino acid sequence KEGED (SEQ ID NO: 8).

17. The compound according to claim 8, said compound having the amino acid sequence IRGIKKTD (SEQ ID NO: 9).

18. The compound according to claim 8, said compound having the amino acid sequence KEGEDGIRGIKKTD (SEQ ID NO: 10).

19. The compound according to claim 8, said compound having the amino acid sequence DKNDE (SEQ ID NO: 11).

20. The compound according to claim 8, said compound having the amino acid sequence TVQARNSIVNAT (SEQ ID NO: 12).

21. The compound according to claim 8, said compound having the amino acid sequence SIHLKVFAK (SEQ ID NO: 13).

22. The compound according to claim 8, said compound having the amino acid sequence LSNNYLQIR (SEQ ID NO: 14).

23. The compound according to claim 8, said compound having the amino acid sequence RFIVLSNNYLQI (SEQ ID NO: 15).

24. The compound according to claim 8, said compound having the amino acid sequence KKDVRFIVLSNNYLQI (SEQ ID NO: 16).

25. The compound according to claim 8, said compound having the amino acid sequence QEFKEGEDAVIV (SEQ ID NO: 17).

26. The compound according to claim 8, said compound having the amino acid sequence KEGEDAVIVCD (SEQ ID NO: 18).

27. The compound according to claim 8, said compound having the amino acid sequence AFSPNGEKLSPNQ (SEQ ID NO: 40).

28. The compound according to claim 8, said compound having the amino acid sequence AKSVVTAEDGTQSE (SEQ ID NO: 41).

29. Use of one or more compounds as defined in any of the claims 8-28 for the manufacture of a medicament for treatment of a disease wherein modulating differentiation, adhesion, and/or survival of NCAM presenting cells is essential for the treatment.

30. The use of claim 29, wherein the medicament is for treating normal, degenerated or damaged NCAM presenting cells.

31. The use of claim 29, wherein the medicament is for treatment comprising the stimulation of differentiation and/or survival of NCAM presenting cells.

32. The use of claim 29, wherein the medicament is for treating the diseases and conditions of the central and peripheral nervous system, or of the muscles or of various organs.

33. The use of claim 29, wherein the medicament is for treating the diseases or conditions of the central and peripheral nervous system, such as postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, Huntington's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression; for treatment of diseases or conditions of the muscles including conditions with impaired function of neuro-muscular connections, such as after organ transplantation, or such as genetic or traumatic atrophic muscle disorders; or for treatment of diseases or conditions of various organs, such as degenerative conditions of the gonads, of the pancreas such as diabetes mellitus type I and II, of the kidney such as nephrosis and of the heart, liver and bowel.

34. The use of claim 29, wherein the medicament is for treating the postoperative nerve damage, traumatic nerve damage, impaired myelination of nerve fibers, postischaemic, e.g. resulting from a stroke, Parkinson's disease, Alzheimer's disease, dementias such as multiinfarct dementia, sclerosis, nerve degeneration associated with diabetes mellitus, disorders affecting the circadian clock or neuro-muscular transmission, and schizophrenia, mood disorders, such as manic depression.

35. The use of claim 29, wherein the medicament is for promoting the wound-healing.

36. The use of claim 29, wherein the medicament is for treating the cancer.

37. The use of claim 29, wherein the medicament is for preventing the cell death of heart muscle cells, such as after acute myocardial infarction, or after angiogenesis.

38. The use of claim 29, wherein the medicament is for promoting the revascularsation.

39. The use of claim 29, wherein the medicament is for stimulating the ability to learn and/or of the short and/or long-term memory.

40. Use of a crystal of the Ig1-2-3 module of NCAM according to claims 1-6 for the in-silico screening a candidate compound capable of modulating NCAM homophylic adhesion-dependent neural plasticity, cell differentiation and/or survival.

41. A pharmaceutical composition comprising one or more compounds as defined in any of the claims 8-28.

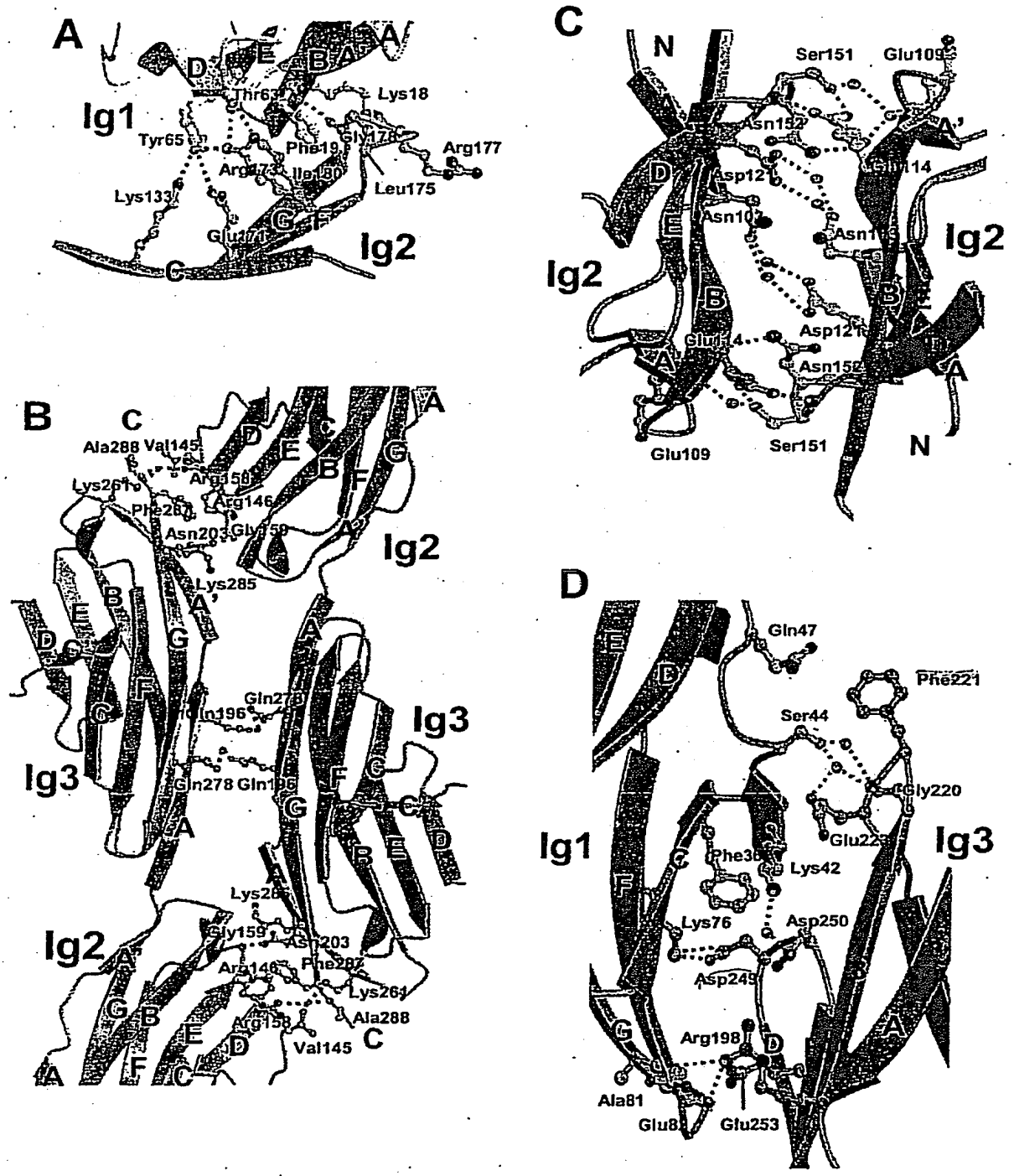
A
Ig1
Ig2
Thr63
Lys18
Tyr65
Phe19
Gly176
Arg177
Arg47
Ile180
Leu175
Lys133
Glu171
B
Ig2
Ig3
Ala288
Val145
Arg158
Lys261
Arg146
Phe287
Asn203
Gly159
Lys285
Gln278
Gln196
C
Ig2
N
Ser151
Glu109
Asn152
Asp12
Asn107
Glu114
Asp121
Asn150
D
Ig1
Ig3
Gln47
Phe221
Ser44
Gly220
Glu223
Phe36
Lys42
Lys76
Asp250
Asp249
Arg198
Ala81
Glu82
Glu253

Figure 5

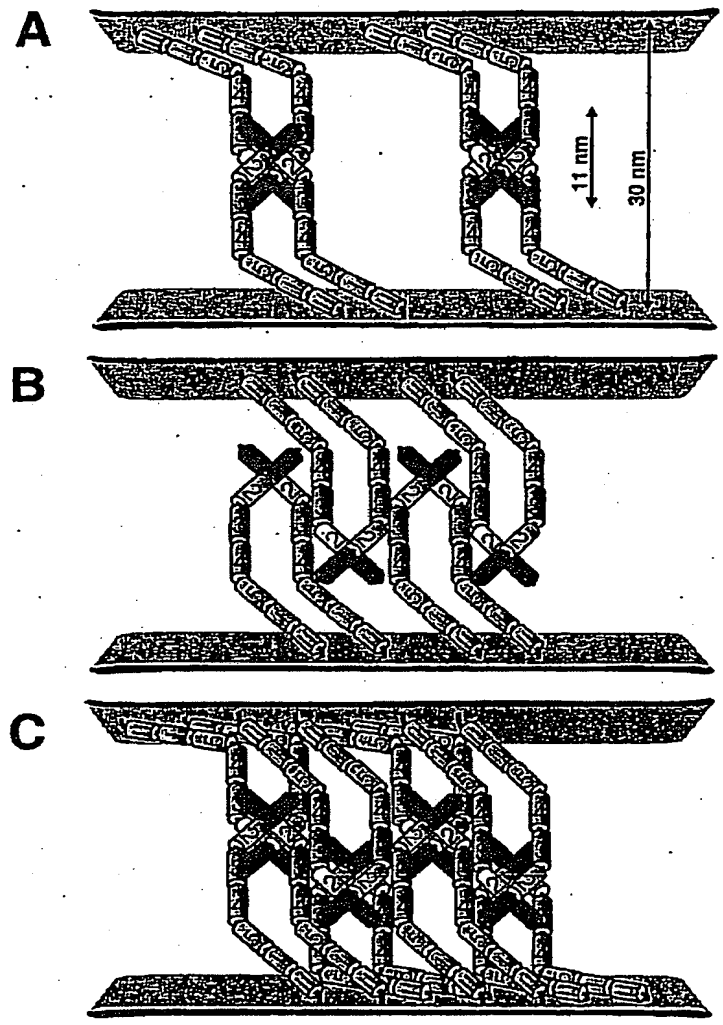

Figure 7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK2004/000659

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K5/00 C07K7/00 C07K14/00 G01N33/68 A61K38/04
A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RAO Y ET AL: "Identification of a peptide sequence involved in homophilic binding in the neural cell adhesion molecule NCAM" JOURNAL OF CELL BIOLOGY, ROCKEFELLER UNIVERSITY PRESS, NEW YORK, US, US, vol. 118, no. 4, August 1992 (1992-08), pages 937-949, XP002118323 ISSN: 0021-9525 cited in the application Abstract; Table IV, Figure 11; Discussion	1-10, 23-60,62

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 April 2005	30 MAY 2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Moonen, P

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE HTTP://WWW 'Online! 2002, KASPER ET AL.: "Extracellular modules of the cell adhesion molecules" XP002315066 retrieved from HTTP://WWW-HASYLAB.DESY.DE/SCIENCE/ANNUAL_REPORTS/2002_REPORT/PART2/CONTRIB/72/7824.PDF the whole document	1-10, 23-60,62
Y	ATKINS A R ET AL: "Solution structure of the third immunoglobulin domain of the neural cell adhesion molecule N-CAM: can solution studies define the mechanism of homophilic binding?" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 311, no. 1, 3 August 2001 (2001-08-03), pages 161-172, XP004469275 ISSN: 0022-2836 cited in the application Abstract; Figure 1; Page 168, first full paragraph -Page 169 left column	1-10, 23-60,62
Y	HUAN Z ET AL: "IMMUNOGLOBULIN SUPERFAMILY PROTEINS: STRUCTURE, MECHANISMS, AND DRUG DISCOVERY" BIOPOLYMERS, NEW YORK, NY, US, vol. 43, no. 5, 1997, pages 367-382, XP001119525 ISSN: 0006-3525 abstract; table I	20-22,49
Y	KASPER CHRISTINA ET AL: "Structural basis of cell-cell adhesion by NCAM" NATURE STRUCTURAL BIOLOGY, vol. 7, no. 5, May 2000 (2000-05), pages 389-393, XP002315064 ISSN: 1072-8368 cited in the application the whole document	20-22,49
A	RONN L C B ET AL: "IDENTIFICATION OF A NEURITOGENIC LIGAND OF THE NEURAL CELL ADHESION MOLECULE USING A COMBINATORIAL LIBRARY OF SYNTHETIC PEPTIDES" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 17, October 1999 (1999-10), pages 1000-1005, XP002902581 ISSN: 1087-0156 abstract	24

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOROKA VLADISLAV ET AL: "Induction of neuronal differentiation by a peptide corresponding to the homophilic binding site of the second Ig module of the neural cell adhesion molecule" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 27, 5 July 2002 (2002-07-05), pages 24676-24683, XP002315062 ISSN: 0021-9258 cited in the application Abstract, Introduction	24
A	KRISTIANSEN L V ET AL: "Homophilic NCAM interactions interfere with L1 stimulated neurite outgrowth" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 464, no. 1-2, 24 December 1999 (1999-12-24), pages 30-34, XP004260716 ISSN: 0014-5793 cited in the application Abstract; Introduction	24
A	JENSEN PETER HOLME ET AL: "Structure and interactions of NCAM modules 1 and 2, basic elements in neural cell adhesion" NATURE STRUCTURAL BIOLOGY, vol. 6, no. 5, May 1999 (1999-05), pages 486-493, XP002315063 ISSN: 1072-8368 cited in the application	
A	WO 00/18801 A2 (ROENN, LARS, CHRISTIAN, B; BOCK, ELISABETH; HOLM, ARNE; OLSEN, MARIANN) 6 April 2000 (2000-04-06) Page 29, SEQ ID NO:26	
X,P	SOROKA VLADISLAV ET AL: "Structure and interactions of NCAM Ig1-2-3 suggest a novel zipper mechanism for homophilic adhesion." STRUCTURE (CAMBRIDGE), vol. 11, no. 10, October 2003 (2003-10), pages 1291-1301, XP002315065 ISSN: 0969-2126 the whole document	1-10, 23-60,62

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/DK2004/000659

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-10, 49
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-10 and 49 are (partially) directed to a method of treatment of or diagnosis applied on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

20-22 completely; 1-10, 23-60 and 62 partially (inventions 1 and 5)

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[X] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: Claims 1-10, 23-60 and 62, partially

Compounds, capable of interacting with the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between Ig1 and Ig3 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.

Invention 2: Claims 1-10, 23-60 and 62, partially

Compounds, capable of interacting with the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between Ig2 and Ig3 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.

Invention 3: Claims 1-10, 23-60 and 62, partially

Compounds, capable of interacting with the NCAM homophylic binding site composed of the Ig1, Ig2 and Ig3 modules and thereby modulating the interaction between two Ig2 modules from two individual NCAM molecules; methods of modulating cells presenting NCAM.

Invention 4: Claims 11-19 and 61

Crystals of a polypeptide comprising the Ig1-Ig2-Ig3 module of NCAM, their use and method of crystallisation.

Invention 5: Claims 20-22 completely; claim 49 partially

Methods for selecting a candidate compound based on a structural model of the Ig1-Ig2-Ig3 modules of NCAM, obtainable eg from the soluble or crystalline polypeptide.

INTERNATIONAL SEARCH REPORT

Information on patent family members

ıtional Application No
PCT/DK2004/000659

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0018801 A2	06-04-2000	AU	761451 B2	05-06-2003
		AU	5727499 A	17-04-2000
		CA	2343975 A1	06-04-2000
		EP	1117680 A2	25-07-2001
		JP	2002525102 T	13-08-2002